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4-14-03
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	§	ATTY DCKT NO: FWLPAT012US
Binie V. Lipps	§	
Frederick W. Lipps	§	
	§	
Serial No.: 09/300,612	§	Art Unit: 1645.
	§	
Filed: 27 April 1999	§	Examiner: Baskar, P.
	§	
For:	§	
ANTI-LTNF FOR IN VITRO ASSAY OF BIOLOGICAL TOXINS	§	

Commissioner of Patents and Trademarks
Washington, D.C. 20231

ATTENTION: Board of Patent Appeals and Interferences

APPELLANT'S BRIEF ON APPEAL

This brief is in furtherance of the Notice of Appeal filed in this case on January 7, 2003.

The fees required under 37 CFR 1.17 (c) and any required petition for extension of time for filing this brief and fees therefor are dealt with in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief is transmitted in triplicate.

REAL PARTY IN INTEREST

The real party in interest is named in the caption of the brief.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 5 and 7-16 are on appeal and are reproduced in the Appendix.

STATUS OF AMENDMENTS

An amendment after final rejection proposing to amend claims 7, 8 and 9 was refused entry. The claims in the application are as set forth in the filing on November 8, 2000 of a continued prosecution application and are reproduced in the appendix.

SUMMARY OF INVENTION

In earlier work, the inventors found that a protein factor contained in opossum serum was capable of neutralizing toxins. They named this factor natural Lethal Toxin Neutralizing Factor, LTNF-n. They further identified the active peptide moiety contained in the protein factor and made synthetic versions of the active moiety, generally designated LTNF-s. Specific examples of LTNF-s include LTNF-15, LTNF-10 and LTNF-5, which contain 15, 10 and 5 amino acids. This earlier work is the subject of US patents 5,576,297 issued November 19, 1996 and 5,744,449, issued April 28, 1998. The instant application, claiming an effective filing date of April 27, 1999, is directed primarily toward the uses of the antibodies formed against the LTNF species of the earlier patents. These antibodies are termed anti-LTNFs.

In the present invention, the inventors found that anti-LTNFs will surprisingly react immunogenically with a vast array of toxins. Since anti-LTNF is made against all or a portion of the LTNF protein, the anti-LTNFs are reacting immunogenically against something they were not made against. This is a surprising result that could not have been predicted by one of ordinary skill. Further, in the present work, the inventors found that the binding affinity between anti-LTNF and toxin is roughly proportional to the lethal dose of the toxin under testing. This is a truly serendipitous discovery, in that it enables an LD_{50}. (the dose required to kill 50% of the test

animals) to be determined without the killing of test animals, for both known and unknown toxins.

Additionally, it is shown in the present work that, while anti-LTNF reacts immunogenically with toxins, (as well as with LTNF), it does not react immunogenically with anti-serums, (such as are used to treat snake envenomation) nor with the immunogenic reaction product between toxins and their specific antiserums. In other words, once the toxin has reacted immunogenically with the anti-serum, it will no longer react immunogenically with the anti-LTNF. This is a still further serendipitous discovery, in that it enables free toxin in a partially neutralized mixture of toxin+antiserum to be assayed, and the neutralizing potency of conventional anti-serums determined, without killing of animals.

It is very surprising that anti-LTNF will react immunologically with toxins as well as with LTNF. Normally, an antibody would be expected to react only with the antigen against which it was produced. While LTNF (the earlier work) was a universal toxin neutralizing factor, enabling toxins to be neutralized, anti-LTNF (this work) is a universal antibody for toxins, enabling toxins to be detected, and, it turns out, also enabling them to be assessed.

ISSUES

ISSUE I: Whether claims 5 and 7-16 are anticipated under 35 USC 102(e) over Lipps et al., US 5,744,449, issued April 28, 1998.

ISSUE II: Whether claim 7 fails to comply with 35 USC 112 because of the recitation "conducting an ELISA binding or ELISA titer."

ISSUE III: Whether claim 8 fails to comply with 35 USC 112 because of the recitation of "food" and further for the recitation of "second antibody."

ISSUE IV: Whether claim 9 fails to comply with 35 USC 112 for indefiniteness in failing to indicate how neutralizing potency is assessed.

GROUPING OF CLAIMS

Concerning Issue I, the claims do not stand or fall together. Lipps discloses that antibodies are made against LTNF-15 and that these antibodies react with both LTNF-15 and LTNF-n. Claims 5 and 7-8 distinguish Lipps on the basis of the step of bringing the anti-LTNF together with a toxin and further by detecting the product of the reaction by ELISA; claims 9-10 distinguish Lipps on the basis of the bringing together of toxin, anti-serum, and anti-LTNF; claims 11-13 distinguish Lipps on the basis that the anti-LTNF is made against a species of LTNF containing 5-10 amino acids; and claims 14-16 distinguish Lipps on the basis of bringing the anti-LTNF together with toxin to cause immunological reaction. Since the claims distinguish over the prior art differently, they do not stand or fall together.

Issue II concerns only claim 7 and the other claims stand separately.

Issue III concerns only claim 8 and the other claims stand separately.

Issue IV concerns only claims 9 and 10. These claims stand or fall together on this issue, whereas the remaining claims stand separately.

ARGUMENTS

ISSUE I

ISSUE I: Whether claims 5 and 7-16 are anticipated under 35 USC 102(e) over Lipps et al., US 5,744,449, issued April 28, 1998 (Lipps '449).

The office action quotes the applicable part of the statute as:

"A person shall be entitled to a patent unless--
(e) the invention was described in a patent granted on an application for patent **by another** filed in the United States before the invention thereof by the applicant for patent,..." (emphasis added).

The inventors in the instant application are Binie V. Lipps and Frederick W. Lipps. The inventors in Lipps '449 are Binie V. Lipps and Frederick W. Lipps, which is identical. Lipps '449 is therefore not "by another". This rejection is therefore improper and it should be reversed as a matter of law.

Additionally, the present claims all distinguish over Lipps '449.

That portion of the disclosure pointed to in the rejection recites:

"...the synthetic LTNF is immunogenic, since mice immunized with it were able to produce specific antibodies, which reacted with both natural and synthetic LTNF..."

The antibodies disclosed in Lipps '449 are made against a synthetic LTNF species, and in the present terminology correspond to anti-LTNF-15. These antibodies are disclosed as reacting with synthetic LTNF (which is the material which they were made against) and natural LTNF, which contains in its structure the peptide sequence against which the antibodies were made.

Claims 5 and 7-8 distinguish Lipps '449 on the basis of the step of bringing the anti-LTNF together with a toxin. In Lipps '449, the anti-LTNF is brought together with LTNF, which is not a toxin, but is rather the nemesis of toxin. Claims 9-10 distinguish Lipps '449 on the basis of the bringing together of toxin, anti-serum, and anti-LTNF. Claims 9-10 do not read on bringing anti-LTNF together with LTNF, and therefore do not read on Lipps '449. Claims 11-13 distinguish Lipps on the basis that the anti-LTNF is made against a species of LTNF containing 5-10 amino acids. In Lipps '449, the anti-LTNF is made against the 15 amino acid species of LTNF, and claims 11-13 excludes this possibility and therefore cannot be anticipated by Lipps '449. Claims 14-16 distinguish Lipps '449 on the basis of bringing the anti-LTNF together with toxin to cause immunological reaction. Since Lipps '449 does not bring anti-LTNF together with toxin, these claims do not read on Lipps '449.

The rejection is therefore improper on the facts, and it should be alternatively reversed on this basis.

ISSUE II

ISSUE II: Whether claim 7 fails to comply with 35 USC 112 because of the recitation "conducting an ELISA binding or ELISA titer."

Claim 7 recites:

7. (twice amended) A process as in claim 5 wherein the anti-LTNF is in a fluid state and the toxin is attached to a plate, said process further comprising

conducting an ELISA binding or ELISA titer on the product of the immunological reaction, and

obtaining a numerical result which is roughly proportional to the toxicity of the at least one biological toxin as determined by animal bioassay.

The rejection states:

"Claim 7 is rejected as being vague and indefinite for the recitation of "conducting an ELISA binding or ELISA titer." It is confusing because normally binding of a protein to its ligand is determined by ELISA. Similarly titer of an antigen or antibody is measured by ELISA. Therefore, applicant is advised to amend the claims to clarify the process more clearly."

Example II of the application discloses reacting anti-LTNF with toxin: The example states:

"Example II -- Immunological reaction of Anti-LTNF with Venom Toxin

Immunological binding of anti-LTNF to venoms by ELISA: The lethal dose was determined by injecting intraperitoneally 0.1 ml of venom in various concentrations in 20g ICR mice. ELISA test was carried in 96 well microplate. The wells of the microplate were coated with 0.1 ml of various concentrations of venom as antigen starting from 100 μg to 0.000564 (564 nanogram) diluted threefold in 0.05 M phosphate buffer saline pH 7.4 (PBS) and incubated for overnight at room temperature. After 18 to 24 hours the plate was washed

three times (3X) with PBS and the plate was blocked with 0.25 ml/well of 3% Teleostean gelatin from cold water fish (Sigma) for 1/2 hour at RT. The plate was washed 3X with PBS and 0.1 ml/well of 10 μg/ml purified mouse anti-LTNF IgG was added. The plate was incubated at 37°C for 1 to 2 hours. And then, the plate was washed 3X with PBS and horseradish peroxidase conjugated with mouse IgG made in goat was added and incubated for 1 hour at 37°C. After which the plate was washed 3X with PBS and reacted with O Phenylenediamine Dihydrochloride (OPD) for color development. The test was read after 1/2 hour visually or preferably on ELISA plate reader. The results are shown in table 2.

Table 2: Immunological binding of anti-LTNF-n to venoms.

Venom	Lethal dose µg	ELISA dose µg	Ratio L/E
Crotalus atrox (Rattlesnake)	300	3.7	81
Naja.n. kaouthia (Thailand cobra)	35	0.1	350
Vipera russelli (Common viper)	90	0.4	225
Oxyuranus scutellatus (Australian taipan)	3.5	0.02	175
Androctonus australis (Scorpion)	15	0.2	75
Astrotia stokesii (Sea snake)	4.0	0.03	133

The results of table 2 show that the toxicity of venoms was roughly proportional to the ELISA binding or ELISA titer. The mouse lethal dose for C. atrox venom was 300 μg and ELISA was 3.7 μg. Mouse lethal dose for sea snake Astrotia stokesii was 4 μg and its ELISA was 0.03 μg. (Emphasis added).

For the purposes of the claimed process, and as supported by Example II, conducting an ELISA binding is equivalent to conducting an ELISA titer. The rejected claim thus particularly points out and distinctly claims the subject matter regarded as the invention. Reversal of the rejection is urged.

ISSUE III

ISSUE III: Whether claim 8 fails to comply with 35 USC 112 is further rejected for indefiniteness for the recitation of food and further for the recitation of "second antibody."

Claim 8 states:

8. (Amended) A process as in claim 5 wherein the biological toxin is contained in a fluid selected from the group consisting of food, blood sera and other body fluid, saliva, milk, and urine and the ELISA is carried out by antigen capture format employing a second antibody.

The rejection states:

> "Claim 8 is rejected as being vague and indefinite for the recitation of "food". Food encompasses both liquids and solids. However, applicant has not shown how biological toxin is obtained from food."
>
> "Claim 8 is rejected as being vague and indefinite for the recitation of "second antibody". What is this antibody? Is it goat anti-mouse IgG or Sheep anti-mouse IgG or what?"

It is stated in the rejection that food encompasses both liquids and solids. This aspect of the rejection is without merit. Claim 8 recites: "...toxin is contained in a fluid selected from the group consisting of food,...". Claim 8 thus only encompasses toxins contained in fluid forms of food and the stated basis for the rejection therefore does not apply. The claim further does not recite obtaining a toxin from food, nor is it necessary to "obtain" the toxin in order to determine its presence. The statement in the rejection "However, applicant has not shown how biological toxin is obtained from food" has nothing to do with the invention as disclosed or claimed. That foods may contain toxins is well known. The claim is directed toward, in one aspect, to detecting, by ELISA using antigen capture format employing a second antibody, the reaction product between any such toxin which may be present and an anti-LTNF. The statement in the rejection "What is this antibody?..." is without merit. The claim recites it as a "second antibody" and the specification makes it clear that the specific selection is relatively unimportant and within the skill of the art. The specification states:

> **ELISA Method in Antigen Capture Format:** ELISA can be done in antigen capture format (also called double sandwich) where anti-LTNF IgG from one species (mouse) is used to capture the toxin and anti-LTNF from another species (rabbit) is used to recognize the toxin. For commercialization, two types of antibodies either polyclonal or monoclonal, to LTNFs, natural, or synthetic, should be used. One for capturing the toxin and other for conjugating with alkaline phosphotase or peroxidase to recognize the toxin as in the antigen capture format.

Toxin detection from foods, sera or other body fluids must be done by antigen capture format. The second antibody can be conjugated directly to alkaline phosphatase or horse radish peroxidase to eliminate one step of washing the plate and to save one-hour incubation period. IgGs from all four anti-LTNFs to: LTNF-n, LTNF-15, LTNF-10 and LTNF-5 were tested in ELISA immunological tests and were found equally good."

The rejected claim thus particularly points out and distinctly claims the subject matter regarded as the invention. Reversal of the rejection is urged

ISSUE IV

ISSUE IV: Whether claim 9 fails to comply with 35 USC 112 for indefiniteness in failing to indicate how neutralizing potency is assessed.

Claim 9 reads as follows:

9. (twice amended) A method for assessing neutralizing potency of an anti-serum against a toxin for which it is specific, said method comprising

determining a neutralizing index given by the difference between

(1) a numerical assay value for a predetermined amount of the toxin in a predetermined amount of a normal serum, and

(2) a numerical assay value for a mixture of the predetermined amount of toxin plus a predetermined amount of the antiserum,

wherein the toxin assay is determined by ELISA test of the toxin plus normal serum;

and the toxin plus anti-serum assay is determined by ELISA test of the mixture of the toxin plus the anti-serum, such mixture containing a reduced amount of free toxin due to neutralization by the anti-serum,

wherein anti-LTNF comprising an antibody made

(1) against natural LTNF, or

(2) against a synthetic peptide consisting of at least five amino acids of the sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

is used as a reagent for the ELISA tests and reacts with free toxin,

wherein the numerical assay values are given by ELISA binding affinity, and

wherein for a given toxin, a higher neutralizing index is indicative of a greater potency for the anti-serum.

The rejection states:

"Claim 9 is rejected as being vague and indefinite because this method is for assessing neutralizing potency of an antiserum. However, there is no step in this method to indicate how that is achieved? There is no bioassay step to indicate neutralization potency."

Claim 9 is directed toward a process as described in Example VI in which venom toxins from various species of snakes were mixed with normal serum or with respective specific anti-serum. The mixtures were incubated at 37° C for 1 hr. to permit neutralization of the toxin by its specific anti-serum. In Example VI, the free toxin (i.e., toxin which has not been neutralized by its antitoxin) is detected in the same manner as in the earlier examples, such as Example II, and this is stated in Example VI. In Example VI, the anti-LTNF utilized in both tests reacts immunologically with the free toxin, but not with neutralized toxin. Coloration will therefore track the amount of toxin reacted by anti-LTNF remaining on the plate, and thus provide an indication of the amount of toxin which was not neutralized by the specific antitoxin. A large difference in coloration in the ELISA titers between the two tests means that a large amount of toxin was neutralized by the specific anti-toxin, and that the specific anti-toxin is therefore highly potent. The claim accurately describes the invention.

As to the contention that the claim fails to set forth a step to indicate how neutralizing potency is assessed, it is pointed out that the claim as a whole does so indicate. Claim 9 recites:

"determining a neutralizing index given by the difference between

(1) a numerical assay value for a predetermined amount of the toxin in a predetermined amount of a normal serum, and

(2) a numerical assay value for a mixture of the predetermined amount of toxin plus a predetermined amount of the antiserum,"

and explains:

"wherein for a given toxin, a higher neutralizing index is indicative of a greater potency for the anti-serum.

The claim thus describes how neutralizing potency is assessed.

The rejection further states:

"The method is totally confusing to the examiner because examiner is viewing antiserum against a toxin being an antibody to a toxin. If that is so, toxin is being bound to antiserum and form a complex and also toxin will bind to an antibody made against natural or synthetic peptide and form complex. Therefore it is not clear how these two complexes are being distinguished from each other and how free toxin is being measured? Is lesser the free toxin in the complex and greater the neutralization as measured by ELISA?"

The contention that the terminology of the claim is confusing is without merit. The claim recites a "method for assessing neutralizing potency of an anti-serum against a toxin **for which it is specific.**" (emphasis added). While anti-LTNF can be considered an anti-serum, **it is not specific,** nor does it constitute a toxin. Anti-serums such as antivenoms are specific. Anti-LTNF is described in the claim as

"comprising an antibody made

(1) against natural LTNF, or

(2) against a synthetic peptide consisting of at least five amino acids of the sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu".

Antibody which is specific against toxin is not made against these materials. The claim thus consistently differentiates between anti-serum and anti-LTNF. As should be clear from the forgoing

discussion, free toxin is not being directly measured in the method. What is being measured is toxin which has reacted with anti-LTNF rather than with anti-serum. ELISA is capable of differentiating these two products.

In view of the foregoing, it is submitted that the 35 USC 112 rejection of claim 9 is improper and should be reversed.

CONCLUSION

In view of the forgoing arguments, reversal of all grounds of rejection is requested.

Respectfully submitted:

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APPENDIX OF CLAIMS INVOLVED IN THE APPEAL

5. (twice amended) A process comprising

(a) bringing together, under in vitro conditions,

(1) an anti-LTNF made

(i) against natural LTNF, or

(ii) against a synthetic peptide consisting of at least five amino acids of the sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

with

(2) at least one biological toxin derived from animal, plant or bacteria,

to cause an immunological reaction which produces a product capable of being detected by ELISA, and

(b) detecting the product of such reaction by ELISA.

7. (twice amended) A process as in claim 5 wherein the anti-LTNF is in a fluid state and the toxin is attached to a plate, said process further comprising

conducting an ELISA binding or ELISA titer on the product of the immunological reaction, and

obtaining a numerical result which is roughly proportional to the toxicity of the at least one biological toxin. as determined by animal bioassay.

8. (Amended) A process as in claim 5 wherein the biological toxin is contained in a fluid selected from the group consisting of food, blood sera and other body fluid, saliva, milk, and urine and the ELISA is carried out by antigen capture format employing a second antibody.

9. (twice amended) A method for assessing neutralizing potency of an anti-serum against a toxin for which it is specific, said method comprising

determining a neutralizing index given by the difference between

(1) a numerical assay value for a predetermined amount of the toxin in a predetermined amount of a normal serum, and

(2) a numerical assay value for a mixture of the predetermined amount of toxin plus a predetermined amount of the antiserum,

wherein the toxin assay is determined by ELISA test of the toxin plus normal serum;

and the toxin plus anti-serum assay is determined by ELISA test of the mixture of the toxin plus the anti-serum, such mixture containing a reduced amount of free toxin due to neutralization by the anti-serum,

wherein anti-LTNF comprising an antibody made

(1) against natural LTNF, or

(2) against a synthetic peptide consisting of at least five amino acids of the sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

is used as a reagent for the ELISA tests and reacts with free toxin,

wherein the numerical assay values are given by ELISA binding affinity, and

wherein for a given toxin, a higher neutralizing index is indicative of a greater potency for the anti-serum.

10. (Twice Amended) A method as in claim 9 wherein the anti-toxins are anti-venoms.

11. (Amended) A composition of matter consisting essentially of an IgG antibody made against a peptide consisting of five to ten amino acids from the N-terminal sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

in the absence of carrier protein molecule.

12. A composition of matter as in claim 11, which is in the form of an immunoglobulin selected from the group consisting of an immunized animal serum, a hybridoma cell culture and a mouse ascitic fluid.

13. (Amended) A composition of matter as in claim 12, which reacts immunologically with a toxin selected from the group consisting of an animal toxin, a plant toxin and bacterial toxin.

14. (Amended) A process comprising contacting, in vitro, a biological toxin with an antibody made against a sequence of at least five amino acids from the N-terminal of the sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

under conditions to cause the biological toxin to react immunologically with said antibody.

15. A process as in claim 14, wherein the novel antibody is made against LTNF having a non-immunological binding with toxins such that its antibody has the property of being able to react immunologically in vitro with a wide range of biological toxins.

16. (Amended) A process as in claim 15 which is carried out according to an ELISA double-sandwich method protocol.